longus extensor digitorum longus muscle retained apparently normal contractile features on P7, but attenuated its vital contractility on P9. On the other hand, spontaneous Ca^{2+} sparks/waves under resting conditions were observed in isolated flexor digitarium breris fibers of *Mg56*-knockout mice on P7-9. Therefore, MG56 essentially contributes to postnatal SR maturation in skeletal muscle, although its contribution to SR Ca^{2+} handling remains to be defined at the molecular level.

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Non-Hypertensive Dosis of Leptin Induce Cardiac Dysfunction and Altered Calcium Handling in Mice

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Leptin, the product of the obese (ob) gene, is an adipocytokine hormone containing 167 amino acids that is involved in appetite suppression and energy expenditure regulation. It is primarily secreted by adipose tissue but it is well accepted that myocardial tissue can also produce leptin, exerting autocrine and paracrine effects. Furthermore, elevated plasma leptin levels are postulated to be an independent risk factor for cardiovascular diseases. Nowadays is increasingly recognized that physiological levels of leptin may be critical for normal cardiomyocyte structure and function whereas very low or very high levels of leptin, may trigger functional and morphological alterations leading to cardiac dysfunction. Here, we have analyzed the effect of non-hypertensive doses of leptin on cardiac function and Ca^{2+} handling in mice. ALZET(tm) osmotic pumps, filled with saline solution or with leptin, were implanted in C57Bl/6J mice and were sacrificed after 3 weeks treatment. The L-type calcium current was registered using whole-cell patch-clamp technique. Intracellular calcium $[Ca^{2+}]$i transients and Ca^{2+} sparks were viewed by confocal microscopy in cardiomyocytes loaded with Fluo-3 AM. Analysis of cardiac function by echocardiography showed that leptin treatment induced a significant reduction of ejection fraction and fractional shortening. Accordingly, $[Ca^{2+}]$i transient amplitude and cell shortening were decreased. Consistent with reduced $[Ca^{2+}]$i transient amplitude, the Ca^{2+} sparks were less intense. However, the SR Ca^{2+} load was unaffected by Leptin treatment. The Ca^{2+} extrusion (decay of caffeine-evoked Ca^{2+} transient) was accelerated, suggesting enhanced function of the Na^{+}/Ca^{2+} exchanger (NCX). Biochemical analyses evidenced an increase in NCX expression and in Ser2808RyR phosphorylation. In conclusion, leptin, at non-hypertensive dose, induces heart dysfunction due, at least in part, to alterations in the NCX and RyR.

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Modeling Mitochondrial Calcium Dynamics in Heart

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Mitochondria are critically important for normal cellular function as they underlie ATP production, initiate apoptosis, and potentially alter cytosolic calcium ($[Ca^{2+}]_i$) signals. In heart, the ends of intermyofibrillar mitochondria (IMF) are in close proximity (~100 nm diffusional distance) to junctional sarcoplasmic reticulum (jSR) which is the source of the elementary SR Ca^{2+} release, the Ca^{2+} spark. With each Ca^{2+} spark or cell-wide $[Ca^{2+}]_i$ transients, the ends of neighboring IMF are exposed to a local microdomain $[Ca^{2+}]$ greater than diastolic $[Ca^{2+}]_i$. The negative inner mitochondrial membrane potential provides a driving force for the uptake of cytosolic Ca^{2+} through the mitochondrial Ca^{2+} uniporter (MCU) into the mitochondrial matrix. Matrix Ca^{2+} ($[Ca^{2+}]_m$) levels are determined by the balance of Ca^{2+} influx via the MCU and Ca^{2+} extrusion via the mitochondrial Na^{+}/ Ca^{2+} exchanger (NCLX). It is unclear, however, if the mitochondria take up enough Ca^{2+} to play a role in buffering cytosolic Ca^{2+} during Ca^{2+} signaling events. A computational approach is ideally suited to investigate mitochondrial Ca^{2+} dynamics since experimental measurements of mitochondrial Ca^{2+} uptake within a cell are very difficult. Here we use a 3D spatial Ca^{2+} diffusion model to assess mitochondrial Ca^{2+} uptake during Ca^{2+} sparks and $[Ca^{2+}]_i$ transients. All key parameters are constrained by recently characterized kinetic information from MCU and NCLX. We find that even in the presence of elevated local $[Ca^{2+}]_i$ microdomains, mitochondrial Ca^{2+} uptake is modest compared to $[Ca^{2+}]_i$ removal via the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) and Na^{+}/Ca^{2+} exchanger (NCX). While unlikely to alter $[Ca^{2+}]_i$ signals significantly, the small changes in $[Ca^{2+}]_m$ estimated by the model should serve to regulate the activity of $[Ca^{2+}]_m$-sensitive enzymes and thus control mitochondrial function.

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Properties of Calcium Transients in Cardiomyocytes with Impaired Insulin Signaling

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Diabetes is a major risk factor for cardiovascular diseases, including cardiac arrhythmias, heart failure and sudden cardiac death. In animal models of diabetes, cardiac calcium handling is severely impaired. However, as diabetes is a multifactorial disease it is difficult to separate the effect of systemic and local factors in disease development. Here, we investigate the effect of locally disrupted cardiac insulin signaling on cardiac calcium handling in a mouse model of diabetic cardiomyopathy.

We measured calcium transients in isolated cardiomyocytes from Cardiac Insulin Receptor Knock-Out (CIRKO) mice. Littermate wild-type (WT) mice were used as controls. Cardiomyocytes were field stimulated and intracellular calcium was monitored using the fluorescent indicator fluo2-LeakRes (34°C, pH_o 7.4, $[Ca^{2+}]_o$ = 1.0 mM). Calcium transient amplitude and decay kinetics were measured at 1 and 5 Hz. While no significant differences were observed between WT and KO cardiomyocytes, there was a tendency towards reduced calcium transient amplitude in the KO group (Amplitude (F/F_0): WT, 1.6 ± 0.7 (n=9); KO, 1.0 ± 0.4 (n=16); p=0.05). Calcium transient decay was similar (tau (ms): WT, 183 ± 44 (n=9); KO: 232 ± 84 (n=17); p=0.15) and frequency-dependent acceleration of decay kinetics was observed in both groups (tau: $p<0.01$ for both WT and KO (1Hz vs 5 Hz)).

In conclusion, at the cellular level the CIRKO mouse model of diabetic cardiomyopathy display a very mild calcium handling phenotype. These findings suggest that impaired cardiac insulin signaling per se plays only a minor role in the contractile dysfunction observed in systemic models of diabetes.

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Sealing of Cardiac T-Tubules Leads to Influx of Trapped Extracellular Ca^{2+}

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Cardiac t-tubules are central players in excitation-contraction coupling. It has been shown that in ventricular myocytes t-tubules can be sealed during various experimental conditions including metabolic inhibition and osmotic stress. Sealing of t-tubules is associated with trapping of extracellular solution inside the myocytes but the fate of various trapped ions and the consequences of potential trans-tubular ion fluxes remain unknown. In this study we aimed to determine the movements of Ca^{2+} during t-tubular sealing induced by resolution of hyposmotic stress. T-tubular and cytosolic concentrations of Ca^{2+} in mouse ventricular myocytes were assessed using Rhod-5N and Fluo-3 Ca^{2+} indicators, respectively. We show that under normal conditions the concentration of Ca^{2+} in sealed t-tubules is below 100 μM. The latter strongly suggests the flux of trapped t-tubular Ca^{2+} into the myocytes during sealing process. Blockade of voltage-dependent Ca^{2+} channels with 10 μM nicardipine led to several fold increase in concentration of Ca^{2+} in sealed t-tubules. Alternatively, release of Ca^{2+} from sarcoplasmic reticulum (SR) in response to 10 mM caffeine also led to restoration of t-tubular Ca^{2+} towards extracellular levels within few seconds. T-tubular sealing under normal ionic conditions led to occasional and sporadic intracellular Ca^{2+} transients, consistent with influx of trapped Ca^{2+}. Accordingly, sealing of t-tubules in the presence of 10 mM caffeine (to prevent Ca^{2+} uptake by SR) was characterized by significant long lasting increase in intracellular Ca^{2+}. The effect was completely abolished in the absence of extracellular Ca^{2+} and significantly reduced in pre-detubulated myocytes. This study shows that sealed t-tubules are capable of highly regulated transport of Ca^{2+} and present a major route for Ca^{2+} influx into myocytes during sealing process.

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Ros in Cardiac Calcium Signaling

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ROS in cardiac calcium signaling Reactive oxygen species (ROS) play important roles in physiologic and pathophysiologic signaling within diverse cells including cardiac myocytes. Here we examine the rapid application of extracellular H_2O_2 (100 uM) to examine how this ROS reagent affects Ca^{2+} signaling in single isolated cardiac ventricular myocytes. Single mouse ventricular myocytes were examined with a confocal microscope and found to have a robust, reversible increase in the Ca^{2+} spark rate. In quiescent cells